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THE USE OF CRYSTALLINE RIBOFLAVIN IN POULTRY RATIONS

by

Alexander R. Robblee

Department of Animal Science

(Poultry Husbandry)

UNIVERSITY OF ALBERTA

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The undersigned hereby certify that they have read and recommend to the Committee on Graduate Studies for acceptance a thesis on "The use of crystalline riboflavin in poultry rations", submitted by A. R. Robblee, B.Sc., in partial fulfilment of the requirements for the degree of Master of Science.

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THE USE OF CRYSTALLINE RIBOFLAVIN IN POULTRY RATIONS

A. R. Robblee

Department of Animal Science

A THESIS

submitted to the University of Alberta
in partial fulfilment of the
requirements for the degree of

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THE USE OF CRYSTALLINE RIBOFLAVIN IN POULTRY RATIONS

INTRODUCTION

The poultry industry, today, has a rôle of major importance in the agricultural economy. This position has been achieved by the gradual increase of the poultry population and the application of improved feeding and breeding programs resulting in increased production.

The demand for increased production performance in the flock has been evident for some time, and has led to the development of an intensified, specialized industry. The change was made possible only by the coincident development of the knowledge of poultry nutrition.

In the past twenty years a vast amount of information has been compiled in the field of poultry nutrition. The dietary requirements of chickens, and to some extent of other poultry, for protein, minerals, and several of the vitamins, has been largely established. As a result of the work already accomplished it is now possible to formulate rations for poultry which may be expected to meet the purposes for which they are intended. However, new problems are constantly arising which must be solved if the efficiency of production is to be maintained or increased.

During the war some products which had been regarded as "necessary" in poultry mashes were not available in sufficient quantity to supply the needs of the poultry producer. This was

the situation with regard to dried milk products which were diverted to human consumption. These products had served as a potent source of riboflavin for poultry mashes as well as a source of other vitamins, minerals and protein. When the amount of milk products used in poultry rations was greatly reduced because of decreasing supplies, riboflavin deficiency symptoms appeared. Fortunately, at this time, the commercial production of pure riboflavin had expanded far enough to make considerable quantities of the vitamin available at a reasonable cost. In view of the serious nature of the situation it was recommended that crystalline riboflavin be incorporated into poultry rations.

During the past few years the use of the pure vitamin in poultry feeds has been extensive and has met with a good deal of success. The widespread use of this vitamin in feeds has resulted in a marked decrease in price until now it is by far the most inexpensive source of riboflavin supplementation in the diet. However, the extent to which this product may be used to replace milk products as a source of riboflavin in poultry rations has been very contentious. Many authorities have claimed that the synthetic product would not successfully replace milk products as a source of the vitamin because of other nutritional factors present in the natural source, while others asserted that pure riboflavin could be used if care were taken to add the nutrients other than riboflavin which are found in milk.

In view of the status of the problem experiments were initiated at the University of Alberta in 1944 to study certain

aspects of the use of crystalline riboflavin in poultry nutrition. The object of this report is to discuss and summarize the results of these experiments. An attempt will be made to indicate whether or not the application of the findings would be of any significance in the economy of a stable poultry industry. A review of the relevant literature precedes the presentation of data on the University of Alberta experiments.

REVIEW OF LITERATURE

History

In 1879 Blyth (1) was successful in separating a substance from milk which he termed "lactochrome". This substance was a yellow-colored compound which was probably an impure riboflavin compound. No biological significance was attached to this compound for many years. In 1913 Osborne and Mendel (2) recognized the presence of a growth promoting, water soluble substance in milk. Although not recognized at that time, it is probable that riboflavin was one of the factors concerned. Emmet and McKim (3) in 1917 noted a difference in the physiological function of vitamin B1 and B2. A further differentiation of the vitamin B complex was suggested by Hauge and Carrick (4) in 1926. They demonstrated that a water soluble, growth promoting factor was needed in addition to the antineuritic substance (thiamin) for satisfactory growth and development of chicks. Hatchability studies by Bethke and Kennard (5), 1928, and

McFarlane, Fulmer and Jukes (6) in 1930 showed that a factor or factors other than the fat soluble vitamins were required for satisfactory results. These factors were found to be associated with green plants, liver, milk and yeast. Norris, Heuser, Wilgus and Ringrose (7, 8) in a further series of studies confirmed and extended the work of Hauge and Carrick and showed that the chick's requirement for this substance was high.

Booher in 1933-34(9, 10) showed that there was a correlation between the vitamin G growth promoting activity of whey and the yellow pigment present.

The term vitamin G at this time referred to a complex of several vitamins. Researches by György (11, 12, 13), Chick et al (14), Hogan and Richardson (15) and Harris (16) definitely established that riboflavin and one or more factors were included in the vitamin G complex.

A great deal of confusion in early work was caused by the different terms used and the misinterpretation of data because of unknown factors. In 1937 it was proposed by the Council on Pharmacy and Chemistry of the American Medical Association (17) that the term d-riboflavin or riboflavin be used in preference to the older terminology vitamin G or vitamin B2.

Chemical and Physical Properties

Riboflavin is a chemical compound of known composition. It occurs in a pure condition as yellow needle-like crystals. It is a yellow-green fluorescent water soluble pigment (10).

The yellow-green fluorescence is at a maximum between a pH of 6.0 and 7.0 (18).

Although this compound is classed as one of the more stable vitamin compounds, it is inactivated in an alkaline medium. Autoclaving in alkaline medium causes rapid inactivation and the rate of destruction is increased by increasing alkalinity. It is relatively stable at ordinary temperatures in acid medium (19). Prolonged heating of feedstuffs at 120°C. also results in the inactivation of this compound (20). It has been demonstrated that riboflavin may be inactivated by irradiation with ultraviolet light (21) or by visible light (13). Supplee, Ansbacher and Bender (22) showed that exposure to natural or artificial light resulted in the loss of growth promoting properties. They found that the fluorescence changes from a yellow-green to a blue. The rays responsible for this inactivation are the radiations with wave lengths between 3100 and 4800 angstroms.

A striking illustration of the general stability of riboflavin was indicated by Barton-Wright, Booth and Pringle (23). Riboflavin assays were run on barley produced over 4000 years ago and obtained from King Tuthankhamen's tomb. This barley was found to contain .85 micrograms of riboflavin per gram which is probably about 2/3 of the amount originally present.

Methods of Assay

The importance of riboflavin in nutrition has led to the development of several means of estimating the content in feedstuffs. The determinations employed may be based on chemical

and physical properties of the compound or on measurement of the physiological activity of the foodstuff concerned.

The basis of the physicochemical methods for the determination of riboflavin is a measurement of the characteristic fluorescence of riboflavin. Several methods have been developed - Supplee et al (24), Weisberg and Levin (25), Hodson and Norris (26).

The biological methods employ experimental animals in the determination. Biological assays using the rat (27) or the chick (28) as test subjects have been developed for this factor. These methods involve the use of riboflavin deficient diets. The diets are supplemented with the foodstuff to be assayed and the additional growth response obtained compared to the positive control allows the calculation of the riboflavin potency.

A microbiological method of assay was developed by Snell and Strong (29) in 1939. This method makes use of the growth response of the organism Lactobacillus casei ε on a medium which is complete in all essential nutrients required for growth except riboflavin. This assay method permits a rapid, accurate determination of riboflavin in a sample. The results obtained check fairly well with the biological and physicochemical methods.

Occurrence

Riboflavin has been found to be widely distributed in plant and animal tissue. Investigations by Mickelsen, Waisman and Elvehjem (30) indicate that the richest sources of riboflavin in the animal body are glandular tissues. Liver and kidney con-

tain more riboflavin than other organs while the content of the muscular tissue is considerably lower.

Milk and dried milk products have been shown to contain a high unitage of riboflavin (31, 26).

Hunt and Bethke (32) have shown that legume and cereal plants offer a good source of riboflavin, especially in the early stages of development. The riboflavin content per unit of weight decreases as the plant matures.

The cereal grains are consistently low in riboflavin content (33).

Physiological Activity

Riboflavin has been shown to be essential for the normal nutrition of a number of species including the rat, poultry, pig, dog and man. The exact role played in the organism has not been fully elucidated but it appears that riboflavin may function in an oxidation-reduction enzyme system in the metabolism of foodstuffs.

Several possible reactions by specific riboflavin containing enzymes have been demonstrated which indicate that it serves as a member of hydrogen transport systems in the oxidation of foodstuffs (34, 35, 36, 37, 38). A detailed consideration of the experimentation which has been carried on with poultry may assist in indicating the essential nature of this vitamin.

Importance of Riboflavin in Poultry Rations

It has been demonstrated that riboflavin is very important in most phases of poultry nutrition. It has been shown to be

essential for growth, prevention of curled-toe paralysis, egg production and hatchability.

Growth - The need of a growth promoting factor in the diet of chicks was indicated by the work of Hauge and Carrick (4) in 1926. It is probable that the factor referred to was riboflavin. This work was confirmed and extended by Norris, Heuser, Wilgus and Ringrose (7), 1930, Bethke, Record and Kennard (39), 1931, and Norris, Heuser, Wilgus and Ringrose (8), 1931. Heiman, Sullivan and Norris (40) in 1934 obtained indirect evidence that the growth promoting factor concerned was a flavin. They found that the ovoflavin content of eggs was related directly to the vitamin G content of the ration of the hen. Norris and his co-workers (41) in 1936 concluded that the growth promoting factor was a flavin. They found there was a close relationship between the rate of growth and the riboflavin level of the ration. This work indicated that the dietary requirements of the chick for this factor is very high, 300 micrograms per 100 grams of feed being required to give maximum weight at six weeks of age.

Hunt, Record and Bethke (42), 1936, confirmed this work and showed a high correlation between vitamin G consumption and rate of growth. The first use of crystalline riboflavin was made by Lepkovsky and Jukes (43) in 1936. They supplemented starter rations deficient in vitamin G with crystalline riboflavin. The growth rate and appearance of the chicks on test was approximately normal. This work was confirmed by Bethke, Record and Wilder (44). Further work by Jukes in 1937 (28) showed that the growth response of chicks on a vitamin G deficient diet was

proportional to the added increments of crystalline lactoflavin when the amounts were small. Bethke and Record (45, 46) found the same relationship.

Culton and Bird (47) compared the addition of dried skim milk and riboflavin to a riboflavin deficient basal ration. The results showed that dried skim milk gave a greater growth response.

Sloan in 1941 (48) found no difference in the growth response obtained from pure riboflavin and dried skim milk in starter rations. Bethke and Record in 1942 (49) demonstrated that synthetic riboflavin and naturally occurring riboflavin were of equal value in the growth of chicks. Clandinin, Woodsworth, Traves and Henderson (50), 1943, used pure riboflavin to replace the riboflavin supplied by 5% buttermilk powder in starter rations. The results of this work indicated that crystalline riboflavin was fully as effective as the naturally occurring riboflavin supplied by the buttermilk powder when the protein supplements were largely of animal origin and of high biological value.

Patrick and Morgan (51) found that crystalline riboflavin gave better results than an equivalent amount supplied in the form of alfalfa meal.

Curled-toe Paralysis - A riboflavin deficiency causes a deficiency disorder in chicks known as curled-toe paralysis. This disorder was noted first by Norris (7, 8) and his associates at Cornell. They suggested that it might be due to a nutritional deficiency. Bethke, Record and Kennard (39) in 1931 confirmed the work done

at Cornell. Bethke, Record and Wilder in 1937 (44) showed that the flavins were concerned in the development of curled-toe paralysis. Stokstad and Manning, 1938, (52) were successful in completely preventing this disorder by the addition of pure riboflavin to the deficient diet.

This was confirmed by Phillips and Engel (53) who also noted that although gross symptoms of curled-toe paralysis may be prevented by a diet containing 300 micrograms per 100 grams of ration, a higher level is required to prevent histopathological symptoms and give complete protection. They also noted that there were two types of deficiency manifestation, one acute, resulting in a paralysis termed neuromalacia, and the other chronic, causing curled-toe paralysis.

Bethke and Record (49) demonstrated that curled-toe paralysis was a manifestation of riboflavin deficiency and that synthetic or naturally occurring riboflavin sources were equally effective in preventing this disorder. They noted that chicks require more riboflavin per unit of feed for the prevention of curled-toe paralysis than for maximum growth.

Egg Production - Riboflavin is required in the ration for normal egg production. Norris et al (41) obtained satisfactory egg production on a diet containing 130 micrograms of riboflavin per 100 grams of ration. Davis, Norris and Heuser (54) found that a level of 100 micrograms per 100 grams of ration was sufficient.

Lepkovsky et al (55) noted that a high level of riboflavin stimulated egg production during the winter months, partic-

ularly following the first adult molt. It was suggested that the level required for normal egg production was greater than that found by Norris and his associates. This work was further borne out by Hunt, Winter and Bethke (56) who found that a level of 150 to 190 micrograms per 100 grams of ration was needed for normal production.

Hatchability - The close relationship between riboflavin and the hatchability of eggs has been shown by many investigators. Bethke, Record and Kennard (57) showed that hatchability was improved by incorporating higher levels of vitamin G in the diet.

Davis, Norris and Heuser (54) showed that there was an increase in embryonic mortality in eggs from hens receiving a deficient diet. Lepkovsky, Taylor, Jukes and Almquist (55) showed that characteristic abnormalities appeared in the riboflavin deficient embryos. Engel, Phillips and Halpin (58) demonstrated the specificity of riboflavin in this regard by injecting 50 micrograms of pure riboflavin into the egg on the first day of incubation. Normal embryonic development resulted. Bauernfeind and Norris (59) showed that the riboflavin content of the egg could be greatly increased by raising the level of riboflavin in the ration.

The level of riboflavin required in the breeding ration is high. Norris and his associates (41) concluded that 230 micrograms per 100 grams of ration are needed to give satisfactory hatchability. This work was substantiated by Hunt, Winter and Bethke (56).

Schumacher and Heuser (60) used crystalline riboflavin to supplement the breeding ration. They found that the administration of crystalline riboflavin resulted in good hatchability. They also noted that hatchability may be influenced very quickly by the riboflavin content of the ration. Clandinin, Traves and Henderson (61) demonstrated that pure riboflavin is effective in replacing the riboflavin supplied by buttermilk powder in certain breeding mashes.

EXPERIMENTS AT THE UNIVERSITY OF ALBERTA

Experiments were designed to study:

I. The riboflavin content of some supplements used in poultry feeds.

II. The effect and feasibility of replacing the riboflavin supplied by buttermilk powder by crystalline riboflavin in chick starter rations.

III. The stability of riboflavin in premixes.

I. The Riboflavin Content of Some Supplements Used in Poultry Feeds.

Status of the Problem

The importance of riboflavin in poultry nutrition has been indicated in the "Review of Literature", and the level required in the feed has been shown to be relatively high. It has been established, also, that the riboflavin content of the basic constituents of the ration, the grains and grain by-products, is consistently low (33). Since the level required in the ration is high an adequate amount must be assured by the addition of riboflavin supplements.

Until a few years ago a large proportion of the riboflavin present in poultry rations was provided by such feedstuffs as dried milk products, liver meal, or certain fermentation products of high riboflavin potency. In the past few years many of

these products have been in short supply and pure riboflavin has been used as a partial replacement. The time, however, will return when these riboflavin bearing feedstuffs become available in quantity in Alberta and it will be well to know just how much riboflavin may be expected to be present in these feedstuffs and how much variation may be expected from one sample to another. It was with these considerations in mind that this particular study was made.

Experimental

Poultry Feed Manufacturers and Dairies were contacted throughout the province and were requested to send in samples of dried milk products, alfalfa meal, meat meal, fish meal, soybean oil meal and cereal grass, at monthly intervals. It was felt that by following this procedure representative samples of ingredients, other than grain and grain by-products, being used in the poultry feed industry in Alberta would be obtained.

The samples received were assayed making use of a microbiological method of assay developed by Snell and Strong (29). This assay depends on the fact that the growth of the organism used, Lactobacillus casei $\mathcal{E}$, is proportional to the amount of riboflavin present in the assay medium and that the amount of acid produced varies directly with the amount of growth that occurs. Titration of the acid produced with 0.1 normal sodium hydroxide is used as a measure of the riboflavin present.

Results

The results of these assays are presented in Table I.

This table shows the number of samples assayed, the range in riboflavin content, the average value, standard deviation and the coefficient of variability. All values are reported on an air dry basis.

TABLE I

Riboflavin Content of Feedstuffs

Sample	No. of Samples	Assay Range γ/gm.	Average Assay γ/gm.	Stand. Dev. S.	Coeff. of Variability
Buttermilk powder	15	32.1 - 42.4	38.4	2.4	6.2
Skimmilk powder	9	18.1 - 21.0	19.4	1.6	8.2
Dried whey	5	20.8 - 23.4	21.9	1.0	4.6
Alfalfa meal	13	4.6 - 12.	9.0	1.9	21.1
Meat meal	9	3.6 - 8.7	5.3	1.9	35.8
Soybean oil meal	10	2.1 - 3.4	3.1	.4	12.9
Fish meal	12	3.2 - 6.6	5.2	1.0	19.2
Cereal grass	1	18.8	18.8	—	—

Discussion

The results obtained in this work indicate that of the feedstuffs assayed the dried milk products are the most dependable sources of riboflavin in the ration. The relative variation of different samples is considerably lower than that found in the other ingredients tested. The coefficient of variability is greater for skimmilk powder than dried whey and buttermilk powder. The riboflavin potency of alfalfa meal is only about half that of dried skimmilk, and samples tend to be much more variable than the milk products. This variability may be due to weather conditions prevailing when the hay is cured, or to the stage of maturity of the plants when the hay is cut. It may be noted that the alfalfa meals assayed were of only average quality. If available, alfalfa meal of excellent quality might be expected to contain somewhat more riboflavin than was found in the samples assayed in this study. The meat meals and fish meals tested had lower riboflavin content than the alfalfa meals. The degree of variability of fish meal was about the same as found for alfalfa meal samples. The samples of meat meal showed the greatest relative variation of the supplements assayed.

The degree of variation in alfalfa meals, fish meals and meat meals makes these products a less reliable source of riboflavin in the ration. In order to obtain a satisfactory measure of the riboflavin potency of these products individual assays would have to be run on individual lots.

Ten samples of soybean oil meal were assayed. The results

show that this product contains a fairly consistent low level of riboflavin.

One sample of cereal grass was obtained and the determination indicated that this product is a very good supplementary source of riboflavin, almost equal to skimmilk powder. Since only one assay was conducted the variability cannot be estimated.

The danger of using average values in formulating poultry rations may be illustrated more clearly if the following situation is considered:

In ration 1A, Table II, the supplementary feeds include alfalfa meal 4%, buttermilk powder 5%, meat meal 10%, and fish meal 5%. If the range of results obtained in these assays is considered the amount of riboflavin contributed to the basal ration by this supplement might vary from 231 micrograms to 380 micrograms per hundred grams of ration.

Summary and Conclusions

Feedstuffs that were obtained from Dairies and Feed Manufacturers in Alberta were assayed for riboflavin by the microbiological method.

Of the feedstuffs assayed, buttermilk powder proved to be the highest in riboflavin, containing almost twice as much of this vitamin as skimmilk powder, whey or cereal grass.

Considerable variation was found in the riboflavin content of different samples of the same feedstuff. The importance of taking this into consideration in connection with compounding rations is indicated.

II. The Effect and Feasibility of Replacing the Riboflavin Supplied by Buttermilk Powder by Crystalline Riboflavin in Chick Starter Rations.

Status of the Problem

The Poultry Feed Industry has been faced in the past few years with a scarcity of riboflavin supplements. Dried milk products in particular have been very scarce and high priced. Many feed manufacturers and poultrymen were forced to reduce the proportion of these ingredients in rations to a minimum. This was followed by a decrease in hatchability and an increase in the incidence of curled-toe paralysis.

Coincident with this shortage the commercial production of synthetic riboflavin had increased. The use of this product as a source of riboflavin in the ration was resorted to with good success. As a rule it was used only as a partial replacement for natural riboflavin supplements. This practice returned a considerable saving in feed costs and resulted in considerable interest in its use. The question was "To what extent can synthetic riboflavin be used in rations as a replacement for natural sources?"

A series of eight experiments was conducted to investigate the effect of using crystalline riboflavin to replace the riboflavin supplied by buttermilk powder in chick starter rations.

Experimental

Day old, unsexed, Single Comb White Leghorn chicks were used in the experiments. At hatching time the chicks were carefully culled and divided into three comparable lots on the basis of a random selection. All chicks were identified with serial wing bands and were weighed individually before being placed in the starting batteries, and at weekly intervals until they were four weeks of age.

The battery room was lighted continuously with white light. Feed and water were kept before the birds at all times. No scratch grain was fed.

Observations were made daily to note the incidence of curled-toe paralysis and the general appearance of the groups.

Groups and Rations

In each experiment the chicks were divided into three comparable groups designated A, B and C.

A - Buttermilk powder (positive control)

B - No buttermilk powder (negative control)

C - Crystalline riboflavin (treatment)

Group A was fed a ration containing the protein combination under consideration as well as 5% buttermilk powder to provide an adequate level of riboflavin. In each experiment the ration fed to Group A constituted what might be considered a practical chick starter.

Group B was the negative control and was fed the same ration as Group A except that the buttermilk powder was removed

and the protein level was adjusted by increasing the level of other protein supplements and grain.

Group C was fed exactly the same ration as group B except that sufficient crystalline riboflavin was added to bring the calculated riboflavin content of the ration to the same level as found in the positive control.

In most cases riboflavin assays were run on the individual feed ingredients of the ration only after the experiments were under way. The actual riboflavin content of the rations as determined by these assays is reported.

The details of the experiments, rations used and results obtained are presented in the following pages.

Experiment 1

Object

To determine the effect of the replacement of buttermilk powder by a substitution involving pure riboflavin in a chick starter ration containing meat meal and fish meal in the ratio of 2:1.

Experimental

Two hundred and forty unsexed, day-old, Single Comb White Leghorn chicks were divided into twelve lots of twenty chicks each. Each lot of twenty chicks occupied a separate compartment of a Petersime battery. Four lots of chicks were placed on each ration.

The formulae of the rations that were compared are shown in Table II.

TABLE II

Starting Rations

Feedstuff	Ration 1A %	Ration 1B %	Ration 1C %
Ground wheat 30) Ground oats 10) Ground barley 10) Ground corn 25)	74.25	76.25	76.25
Buttermilk powder	5.0	--	--
Fish meal	5.0	6.0	6.0
Meat meal	10.0	12.0	12.0
Alfalfa meal	4.0	4.0	4.0
Ground limestone	1.0	1.0	1.0
Iodized salt	0.5	0.5	0.5
Fish oil (1500A 200D)	.25	.25	.25
Manganese sulphate	*	*	*
Riboflavin			**
Riboflavin content in micrograms per 100 grams	386	222	398

* Manganese sulphate was added at the rate of 1/3 lb. per ton.

** Riboflavin was added at the rate of 176 micrograms per 100 grams ration.

1A - Buttermilk powder (positive control)

1B - No " " (negative ")

1C - Crystalline riboflavin (treatment)

Results

The results obtained from the three rations will be found in Table III.

TABLE III

Average Weight and Per Cent Incidence of Curled-toe Paralysis at Four Weeks of Age

Ration	Sex	Number of Chicks	Average Weight (grams)	Incidence of Curled-toe Paralysis %
1A	Male	47	341.9	0
	Female	32	305.5	
1B	Male	45	338.9	8
	Female	34	296.3	
1C	Male	37	330.7	0
	Female	41	298.5	

Observations

(1) The chicks on all rations grew very well.

(2) Eight per cent of the chicks fed ration 1B developed curled-toe paralysis.

(3) No cases of curled-toe paralysis were observed on rations 1A and 1C.

(4) There was no difference in the general appearance of lots A and C.

Discussion

There was no significant difference in the weights of the three groups at four weeks of age. The fact that some of

the chicks on ration 1B developed curled-toe paralysis indicates that this ration was low in riboflavin.

The addition of riboflavin to ration 1C was effective in preventing the appearance of this disorder. Since there was no difference in the growth rate of the three groups, the absence of curled-toe paralysis in group 1C serves as a criterion of the effectiveness of the pure riboflavin supplementation.

Experiment 2

Object

To determine the effect of the replacement of buttermilk powder by a substitution involving pure riboflavin in a chick starter ration containing meat meal, fish meal, and soybean oil meal in the ratio 1:1:1.

Experimental

Two hundred and forty unsexed, day-old, Single Comb White Leghorn chicks were divided into three groups as in Experiment 1.

The formulae of the rations that were compared are shown in Table IV.

TABLE IV

Starting Rations

Feedstuff	Ration 2A %	Ration 2B %	Ration 2C %
Ground wheat 30) Ground oats 10) Ground barley 10) Ground corn 25)	73.25	74.95	74.95
Buttermilk powder	5.0	--	--
Fish meal	5.2	6.3	6.3
Meat meal	5.2	6.3	6.3
Soybean oil meal	5.2	6.3	6.3
Alfalfa meal	4.0	4.0	4.0
Ground limestone	1.4	1.4	1.4
Iodized salt	.5	.5	.5
Fish oil (1500A 200D)	.25	.25	.25
Manganese sulphate	*	*	*
Riboflavin			**
Riboflavin content in micrograms per 100 grams	376	210	386

* Manganese sulphate was added at the rate of 1/3 lb. per ton.

** Riboflavin was added at the rate of 176 micrograms per 100 grams ration.

2A - Buttermilk powder (positive control)

2B - No " " (negative ")

2C - Crystalline riboflavin (treatment)

Results

The results obtained are shown in Table V.

TABLE V

Average Weight and Per Cent Incidence of Curled-toe Paralysis at Four Weeks of Age

Ration	Sex	Number of Chicks	Average Weight (grams)	Incidence of Curled-toe Paralysis %
2A	Male	37	336.8	0
	Female	43	305.1	
2B	Male	42	331.1	8
	Female	36	289.3	
2C	Male	42	329.3	0
	Female	38	293.0	

Statistical Analysis

Variance Due To	DF	Sum Squares	Mean Square	F	1%	5%
Rations	2	3253	1626.5	1.66	4.71	3.04
Sex	1	76514	76514	78.04^{xx}	6.76	3.89
Rations x Sex	2	4389	2194.5	2.24	4.71	3.04
Error	233	228423	980.4			
Total	238	312579				

Minimum Significant Difference

Sex = 8.1 grams

Observations

(1) The growth of the chicks on all the rations was good.

(2) Eight percent of the chicks on ration 2B developed curled-toe paralysis.

(3) There were no cases of curled-toe paralysis observed on rations 2A and 2C.

(4) There was no difference in the appearance of the chicks on rations 2A and 2C.

Discussion

The results of this experiment were substantially the same as in the first experiment. There was no significant difference in the weights of the three groups at four weeks of age. Curled-toe paralysis was the only indication that the ration 2B was deficient in riboflavin. The substitution used in this experiment proved quite satisfactory.

The results of other investigators (41, 42) would indicate that the level of riboflavin in ration 2B would not support maximum growth. It may be possible that protein combinations of high biological value exert a sparing action on the amount of riboflavin required for growth. Abnormally high intestinal synthesis of riboflavin in the chicks used in these experiments may also be advanced as a possible explanation, although the work of Lamoreux and Schumacher (62) suggests that such synthesis is not likely to be significant. It was decided in the following experiment to provide only one source of animal protein in place of combinations of protein.

Experiment 3

Object

To determine the effect of the replacement of buttermilk powder by a substitution involving crystalline riboflavin in a chick starter containing meat meal as the source of animal protein.

Experimental

Two hundred and four unsexed, day-old, Single Comb White Leghorn chicks were divided into twelve lots of seventeen chicks each. Each lot of seventeen chicks occupied a separate section of a Petersime battery. Four lots of chicks were placed on each ration.

Rations

The formulae of the rations used are shown in Table VI.

TABLE VI

Starting Rations

Feedstuff	Ration 3A %	Ration 3B %	Ration 3C %
Ground wheat 30) Ground oats 10) Ground barley 10) Ground corn 25)	70.75	72.85	72.85
Buttermilk powder	5.0	--	--
Meat meal	18.5	21.4	21.4
Alfalfa meal	4.0	4.0	4.0
Ground limestone	1.0	1.0	1.0
Iodized salt	0.5	0.5	0.5
Fish oil (1500A 200D)	0.25	0.25	0.25
Manganese sulphate	*	*	*
Riboflavin			**
Riboflavin content in micrograms per 100 grams	359	205	359

* Manganese sulphate was added at the rate of 1/3 lb. per ton.

** Riboflavin was added at the rate of 154 micrograms per 100 grams ration.

3A - Buttermilk powder (positive control)

3B - No " " (negative ")

3C - Crystalline riboflavin (treatment)

Results

The results obtained from these three rations will be found in Table VII.

TABLE VII

Average Weight and Per Cent Incidence of Curled-toe Paralysis at Four Weeks of Age

Ration	Sex	Number of Chicks	Average Weight (grams)	Incidence of Curled-toe Paralysis %
3A	Male	51	280.0	0
	Female	17	251.2	
3B	Male	49	195.4	27
	Female	18	182.7	
3C	Male	48	279.9	0
	Female	18	242.2	

Statistical Analysis

Variance Due To	DF	Sum Squares	Mean Square	F	1%	5%
Rations	2	280948	140474	15.35^{xx}	4.71	3.04
Sex	1	28574	28574	31.22^{xx}	6.76	3.89
Rations x Sex	2	2702	1351	1.48	4.71	3.04
Error	233	213219	915.1			
Total	238	525443				

Minimum Significant Difference

Rations = 10.5

Sex = 11.7

Observations

(1) Growth of the chicks on ration 3B was significantly lower than that obtained on rations 3A and 3C.

(2) Twenty-seven per cent incidence of curled-toe paralysis was noted in the chicks on ration 3B.

(3) Growth obtained on rations 3A and 3C was only fair. The average weight of the chicks was about 90 grams less than on the corresponding rations in Experiments 1 and 2.

(4) The chicks on ration 3A had a better appearance than those on 3C. There was a tendency to roughness of feathering in lot 3C.

(5) No cases of curled-toe paralysis were noted on rations 3A and 3C.

Discussion

The high incidence of curled-toe paralysis on ration 3B shows that this ration is low in riboflavin content for these chicks. In addition the growth rate of these chicks is significantly lower than that of the other two lots. There was no significant difference in the growth of 3A and 3C and no cases of curled-toe paralysis were noted. It would appear that with the protein adjustment used in this experiment the addition of synthetic riboflavin to ration 3C was fairly effective in replacing the riboflavin contributed by the 5% buttermilk powder of ration 3A.

The roughness noted in lot 3C may have been due to a lowering of the protein quality of the ration due to the omission of

the buttermilk powder. Meat meal, as a source of protein, is lower in biological value than buttermilk powder, and as a result, ration 3C could not be expected to be comparable to 3A. It was decided in the next experiment to use fish meal in making the protein adjustment in the B and C rations, since fish meal, as a source of protein, has a biological value equal or superior to buttermilk powder.

Experiment 4

Object

To determine the effect of the replacement of buttermilk powder by a substitution involving crystalline riboflavin in a chick starter in which meat meal is the main source of animal protein, fish meal being used to replace the protein supplied by buttermilk powder.

Experimental

Two hundred and forty day-old, Single Comb White Leghorn unsexed chicks were divided into six lots of forty chicks each. Each lot of forty chicks occupied a separate double compartment of a Petersime battery. Duplicate lots of chicks were placed on each ration.

Rations

Formulae of the starting rations that were compared are shown in Table VIII.

TABLE VIII

Starting Rations

Feedstuff	Ration 4A %	Ration 4B %	Ration 4C %
Ground wheat 30) Ground oats 10) Ground barley 10) Ground corn 25)	70.75	73.75	73.75
Buttermilk powder	5.0	--	--
Meat meal	18.5	18.5	18.5
Alfalfa meal	4.0	4.0	4.0
Ground limestone	1.0	1.0	1.0
Iodized salt	.5	.5	.5
Fish meal	--	2.0	2.0
Fish oil (1500A 200D)	.25	.25	.25
Manganese sulphate	*	*	*
Riboflavin			**
Riboflavin content in micrograms per 100 grams	468	271	421

* Manganese sulphate was added at the rate of 1/3 lb. per ton.

** Riboflavin was added at the rate of 150 micrograms per 100 grams ration.

4A - Buttermilk powder (positive control)

4B - No " " (negative ")

4C - Crystalline riboflavin (treatment)

The positive control is the same as in Experiment 3. The protein adjustment in rations 4B and 4C was made with fish meal and grain instead of with meat meal and grain as was the case in the previous experiment.

Results

The results obtained are presented in Table IX.

TABLE IX

Average Weight and Per Cent Incidence of Curled-toe Paralysis at Four Weeks of Age

Ration	Sex	Number of Chicks	Average Weight (grams)	Incidence of Curled-toe Paralysis %
4A	Male	41	278.8	0
	Female	37	242.6	
4B	Male	43	253.6	8
	Female	37	218.8	
4C	Male	43	285.3	0
	Female	33	258.5	

Observations

(1) Growth response on ration 4B was significantly lower than that obtained on the other two rations.

(2) An 8% incidence of curled-toe paralysis was observed in the chicks on this ration.

(3) Growth obtained on ration 4A and 4C was satisfactory.

(4) There was no difference in appearance of the chicks in the positive control and the treatment.

(5) No cases of curled-toe paralysis were noted in either lots 4A or 4C.

Discussion

The chicks on ration 4B showed an eight per cent incidence of curled-toe paralysis indicating that this ration was low in riboflavin content for these chicks. The average weight of this group at four weeks of age was significantly lower than that obtained on groups 4A and 4C.

The average weight reached on ration 4A, which is the same formula as ration 3A, compared favorably with the preceding experiment. The use of fish meal in the negative control and treatment groups resulted in a noticeable increase in the growth rate of these groups as compared with the same groups in the previous experiment.

The percentage of curled-toe paralysis which occurred on ration 4B (8%) was considerably lower than on ration 3B (27%). This decrease was probably due to the higher riboflavin content of ration 4B. Ration 4B contained 271 micrograms per 100 grams of ration compared with 205 in ration 3B. The increased level in ration 4B is an example of the danger of using average values in estimating the riboflavin content of rations.

The chicks on ration 4C showed no curled-toe paralysis and made as good growth as the positive control chicks. The addition of pure riboflavin to ration 4C was therefore effective in replacing the riboflavin contributed by the 5% buttermilk powder of the positive control ration under the conditions of the experiment.

Experiment 5

Object

To determine the effect of the replacement of buttermilk powder by a substitution involving crystalline riboflavin in chick starter containing fish meal as a source of animal protein.

Experimental

Two hundred and four day-old, unsexed, Single Comb White Leghorn chicks were divided into six lots of thirty-four chicks each. Each lot of thirty-four chicks occupied a separate double compartment of a Petersime battery. Duplicate lots of chicks were placed on each ration.

Rations

Formulae of starting rations that were compared are shown in Table X.

TABLE X

Starting Rations

Feedstuff	Ration 5A %	Ration 5B %	Ration 5C %
Ground wheat 30) Ground oats 10) Ground barley 10) Ground corn 25)	76.25	79.25	79.25
Buttermilk powder	5.0	--	--
Fish meal	13.0	15.0	15.0
Alfalfa meal	4.0	4.0	4.0
Ground limestone	1.0	1.0	1.0
Iodized salt	0.5	0.5	0.5
Fish oil (1500A 200D)	0.25	0.25	0.25
Manganese sulphate	*	*	*
Riboflavin			**
Riboflavin content in micrograms per 100 grams	405	258	405

* * Manganese sulphate was added at the rate of 1/3 lb. per ton.

** Riboflavin was added at the rate of 147 micrograms per 100 grams ration.

5A - Buttermilk powder (positive control)

5B - No " " (negative ")

5C - Crystalline riboflavin (treatment)

Results

The results obtained from the three rations will be found in Table XI.

TABLE XI

Average Weight and Per Cent Incidence of Curled-toe Paralysis at Four Weeks of Age

Ration	Sex	Number of Chicks	Average Weight (grams)	Incidence of Curled-toe Paralysis %
5A	Male	52	356.0	0
	Female	16	305.9	
5B	Male	49	309.4	20
	Female	17	292.4	
5C	Male	50	345.9	0
	Female	18	312.8	

Statistical Analysis

Variance Due To	DF	Sum Squares	Mean Square	F	1%	5%
Rations	2	58358	29179	16.07^{xx}	4.71	3.04
Sex	1	43365	43365	23.88^{xx}	6.76	3.89
Rations x Sex	2	5552	2776	1.53	4.71	3.04
Error	197	357662	1815.54			
Total	202	464937				

Minimum Significant Difference

Rations = 14.8

Sex = 16.8

Observations

(1) The growth rate obtained on rations 5A and 5C was very good.

(2) No difference was noted in the general appearance of the chicks in the positive control and treatment groups.

(3) No cases of curled-toe paralysis were noted on ration 5A or 5C.

(4) Growth and general appearance of chicks on ration 5B was considerably poorer than that of those on rations 5A and 5C.

(5) A twenty per cent incidence of curled-toe paralysis was noted in the chicks on ration 5B.

Discussion

The chicks on ration 5B showed a high incidence of curled-toe paralysis. Further, the weight obtained in this group was significantly lower than in groups 5A and 5C. This indicates that this ration is deficient in riboflavin for these chicks.

There were no cases of curled-toe paralysis observed in lots 5A and 5C and no significant difference was found in the average weights of these groups at four weeks of age. The addition of crystalline riboflavin to ration 5C was therefore effective in replacing the riboflavin contributed by the buttermilk powder of the positive control ration when an appropriate protein adjustment was made.

The use of fish meal as the main source of supplementation in the rations resulted in higher average weights than in any of the preceding experiments. This serves to indicate that fish meal is a superior source of protein in chick rations.

Experiment 6

Object

To determine the effect of the replacement of buttermilk powder by a substitution involving crystalline riboflavin in a chick starter containing a high percentage of soybean oil meal and a low percentage of meat meal when the protein adjustment in the negative control and treatment is made with meat meal.

Experimental

Two hundred and forty unsexed, day-old, Single Comb White Leghorn chicks were divided into three groups as in Experiment 1.

Rations

Formulae of the starting rations that were compared are shown in Table XII.

TABLE XII

Starting Rations

Feedstuff	Ration 6A %	Ration 6B %	Ration 6C %
Ground wheat 30) Ground oats 10) Ground barley 10) Ground corn 25)	64.75	66.45	66.45
Buttermilk powder	5.0	--	--
Soybean oil meal	20.	20.	20.
Meat meal	3.5	6.8	6.8
Alfalfa meal	4.0	4.0	4.0
Iodized salt	.5	.5	.5
Ground limestone	1.5	1.5	1.5
Bonemeal	.5	.5	.5
Fish oil (1500A 200D)	.25	.25	.25
Manganese sulphate	*	*	*
Riboflavin			**
Riboflavin content in micrograms per 100 grams	392	206	366

* Manganese sulphate was added at the rate of 1/3 lb. per ton.

** Riboflavin was added at the rate of 160 micrograms per 100 grams ration.

6A - Buttermilk powder (positive control)

6B - No " " (negative ")

6C - Crystalline riboflavin (treatment)

Results

The results obtained from the feeding trials will be found in Table XIII.

TABLE XIII

Average Weight and Per Cent Incidence of Curled-toe Paralysis at Four Weeks of Age

Ration	Sex	Number of Chicks	Average Weight (grams)	Incidence of Curled-toe Paralysis %
6A	Male	47	318.9	0
	Female	31	295.5	
6B	Male	45	223.8	38
	Female	31	213.4	
6C	Male	40	291.4	0
	Female	40	270.6	

Statistical Analysis

Variance Due To	DF	Sum Squares	Mean Square	F	1%	5%
Rations	2	325675	162837.5	177.69^{xx}	4.71	3.04
Sex	1	17340	17340	18.92^{xx}	6.76	3.89
Rations x Sex	2	3382	1691	1.84	4.71	3.04
Error	229	209850	916.4			
Total	234	556247				

Minimum Significant Difference

Rations = 9.8 grams

Sex = 8.5 grams

Observations

(1) Curled-toe paralysis developed in 38% of the chicks on ration 6B.

(2) The general appearance of chicks on ration 6B was considerably poorer than that of those on rations 6A and 6C.

(3) No cases of curled-toe paralysis were noted on rations 6A and 6C.

(4) Growth obtained on rations 6A and 6C was good.

(5) No difference in appearance was noted on rations 6A and 6C.

Discussion

In this experiment a large part of the protein supplement was supplied by soybean oil meal. Meat meal and buttermilk powder were the only sources of animal protein in the positive control. In the other groups buttermilk powder was removed and the percentage of meat meal was increased to adjust for the protein removed.

A very high incidence of curled-toe paralysis was noted on ration 6B which indicates a riboflavin deficiency in the ration. The growth rate in this lot was significantly lower than that obtained in the other groups.

No cases of curled-toe paralysis occurred in lots 6A and 6C, but the weight of lot 6C was significantly lower than that of 6A. Although the addition of riboflavin to ration 6C was effective in preventing curled-toe paralysis, the difference in the weight of lots 6A and 6C suggests that the protein adjustment

was not adequate to provide for normal growth. In Experiment 3 also there was an indication that the replacement of the protein of buttermilk powder by meat meal was not too satisfactory. The use of meat meal alone as a protein adjustment in a replacement of this nature cannot be recommended on the basis of the results of this experiment and Experiment 3.

Experiment 7

Object

In Experiment 6 it was found that the use of meat meal as a replacement for the protein of buttermilk powder was not satisfactory. It had been noted previously in Experiment 4 that when fish meal was used for this purpose much more satisfactory results had been obtained. This experiment was designed to study the effect of the replacement of buttermilk powder by a substitution involving crystalline riboflavin in a chick starter containing a high percentage of soybean oil meal and a low percentage of meat meal, when the protein adjustment in the negative control and treatment is made with fish meal.

Experimental

Two hundred and forty day-old, Single Comb White Leghorn unsexed chicks were divided into three groups as in Experiment 4.

Rations

Formulae of the starting rations that were compared are shown in Table XIV.

TABLE XIV

Starting Rations

Feedstuff	Ration 7A %	Ration 7B %	Ration 7C %
Ground wheat 30) Ground oats 10) Ground barley 10) Ground corn 25)	64.75	67.25	67.25
Buttermilk powder	5.0	--	--
Soybean oil meal	20.0	20.0	20.0
Meat meal	3.5	3.5	3.5
Fish meal	--	2.5	2.5
Alfalfa meal	4.0	4.0	4.0
Iodized salt	.5	.5	.5
Limestone	1.5	1.5	1.5
Bonemeal	.5	.5	.5
Fish oil (3000A 400D)	.25	.25	.25
Manganese sulphate	*	*	*
Riboflavin			**
Riboflavin content in micrograms per 100 grams	392	209	369

* Manganese sulphate was added at the rate of 1/3 lb. per ton.

** Riboflavin was added at the rate of 160 micrograms per 100 grams ration.

7A - Buttermilk powder (positive control)

7B - No " " (negative ")

7C - Crystalline riboflavin (treatment)

Results

The results obtained from the three rations will be found in Table XV.

TABLE XV

Average Weight and Per Cent Incidence of Curled-toe Paralysis at Four Weeks of Age

Ration	Sex	Number of Chicks	Average Weight (grams)	Incidence of Curled-toe Paralysis %
7A	Male	46	306.2	0
	Female	34	280.1	
7B	Male	40	236.4	38
	Female	38	230.5	
7C	Male	34	320.4	0
	Female	44	277.7	

Statistical Analysis

Variance Due To	DF	Sum Squares	Mean Square	F	1%	5%
Rations	2	202105	101052.5	13.48	99.01	19.00
Sex	1	33929	33929	4.52	98.49	18.51
Rations x Sex	2	14996	7498	5.31**	4.71	3.04
Error	231	326213	1412.2			
Total	236	577243				

Observations

(1) Curled-toe paralysis developed in 38% of the chicks on ration 7B.

(2) The general appearance of the chicks on ration 7B was poorer than on rations 7A and 7C.

(3) The growth obtained with diets 7A and 7C was very good.

(4) No difference in general appearance was noted on rations 7A and 7C. Further, no cases of curled-toe paralysis were noted in these groups.

Discussion

The weight of the chicks on ration 7B was decidedly lower than on rations 7A and 7C. The chicks on the negative control ration showed a high incidence of curled-toe paralysis. The occurrence was comparable to that noted in Experiment 6.

No significant difference was observed in the growth rate or general appearance of the chicks on ration 7A and 7C. The use of fish meal in the negative control and treatment rations resulted in a definite increase in the weight of these chicks at four weeks of age as compared to the same groups in Experiment 6.

The replacement of buttermilk powder in the control ration by a substitution involving crystalline riboflavin and fish meal proved effective under the conditions of the experiment.

Experiment 8

Object

To determine the effect of the replacement of buttermilk powder by a substitution involving crystalline riboflavin in a chick starter containing soybean oil meal and fish meal in the proportion 8:1.

Experimental

Two hundred and eighty day-old, Single Comb White Leghorn unsexed chicks were divided into seven lots of forty chicks each. Each lot of forty chicks occupied a separate double compartment of a Petersime battery. Duplicate lots of chicks were placed on two of the rations, and triplicate lots on the remaining ration.

Rations

Formulae of the starting rations that were compared are shown in Table XVI.

TABLE XVI

Starting Rations

Feedstuff	Ration 8A %	Ration 8B %	Ration 8C %
Ground wheat 30) Ground oats 10) Ground barley 10) Ground corn 25)	65.25	67.75	67.75
Buttermilk powder	5.0	--	--
Soybean oil meal	20.0	20.0	20.0
Fish meal	2.5	5.0	5.0
Alfalfa meal	4.0	4.0	4.0
Iodized salt	.5	.5	.5
Ground limestone	1.5	1.5	1.5
Fish oil (3000A 400D)	.25	.25	.25
Bonemeal	1.0	1.0	1.0
Manganese sulphate	*	*	*
Riboflavin			**
Riboflavin content in micrograms per 100 grams	358	212	352

* Manganese sulphate was added at the rate of 1/3 lb. per ton.

** Riboflavin was added at the rate of 140 micrograms per 100 grams ration.

8A - Buttermilk powder (positive control)

8B - No " " (negative ")

8C - Crystalline riboflavin (treatment)

Results

The results obtained from the three rations will be found in Table XVII.

TABLE XVII

Average Weight and Per Cent Incidence of Curled-toe Paralysis at Four Weeks of Age

Ration	Sex	Number of Chicks	Average Weight (grams)	Incidence of Curled-toe Paralysis %
8A	Male	74	318.7	0
	Female	44	295.3	
8B	Male	44	253.3	27
	Female	35	256.4	
8C	Male	39	319.5	0
	Female	41	300.6	

Statistical Analysis

Variance Due To	DF	Sum Squares	Mean Square	F	1%	5%
Rations	2	172320	86160	22.89**	99.01	19.00
Sex	1	14861	14861	3.95	98.49	18.51
Rations x Sex	2	7529	3764.5	3.53*	4.71	3.04
Error	272	290299	1067.3			
Total	277	485009				

Minimum Significant Difference

Rations = 29.3

Observations

(1) A 27% incidence of curled-toe paralysis was noted on ration 8B.

(2) The general appearance of this group was much poorer than that of the other groups.

(3) There was no difference observed in the appearance of groups 8A and 8C.

(4) No cases of curled-toe paralysis were noted in groups 8A and 8C.

Discussion

The weight of the chicks on ration 8B was significantly lower than that of those on rations 8A and 8C. The chicks on ration 8B also showed a high incidence of curled-toe paralysis which indicates that the ration was deficient in riboflavin for these chicks. There was no significant difference in the weight of the chicks on rations 8A and 8C and no cases of curled-toe paralysis were observed in either lot. The replacement of the buttermilk powder by a substitution involving riboflavin and fish meal proved effective under the conditions of the experiment.

General Discussion

The omission of buttermilk powder or other milk products from chick starter rations lowers both the vitamin and protein content of the resulting ration. The deficiency may be exhibited in two ways:

(1) A decrease in the rate of growth.

(2) The occurrence of curled-toe paralysis.

The decreased growth rate may be due to either a riboflavin deficiency or a lowering of the quality of the protein in the ration, or to a combination of both factors. Curled-toe paralysis is the specific manifestation of a riboflavin deficiency.

In the replacement of buttermilk powder in the ration a suitable adjustment should supply the same unitage of riboflavin and protein of equivalent biological value to that contributed by the buttermilk powder. In the experiments conducted the riboflavin adjustment in the treatment group was made with pure riboflavin. The protein replacement of buttermilk powder was made with a number of protein supplements.

The addition of pure riboflavin to the treatment rations prevented the appearance of curled-toe paralysis in every case. It would appear, therefore, that the pure product was a satisfactory replacement for the riboflavin supplied in buttermilk powder.

The protein replacement was made with a number of protein supplements. In Experiments 1 and 2 the adjustment was made by increasing the percentage of the protein combinations in the

ration. The growth obtained in the treatment groups was comparable to that obtained in the positive control. This would indicate that these combinations constituted a satisfactory replacement for the protein of buttermilk powder. In these experiments the average weight of the negative control groups equalled that of the positive controls. This effect is puzzling but it may be possible that the ration used has an influence on the riboflavin requirement or on intestinal synthesis. Further investigations, however, would have to be conducted to prove or disprove this explanation.

In Experiments 4, 5, 7 and 8, fish meal was used to make the protein adjustments. No significant difference in the weight of the positive control and the treatment group was found. This would indicate that fish meal is a satisfactory source of protein to replace buttermilk powder.

The use of meat meal as a substitute for the protein of buttermilk powder in Experiments 3 and 6 showed that this product is not satisfactory for this purpose.

The rations containing a high percentage of soybean meal gave quite satisfactory growth in the positive control groups and in the treatment groups when fish meal was used to replace the protein of buttermilk powder. The growth rate was significantly lower when meat meal instead of fish meal was used to replace the protein of buttermilk powder in Experiment 6. The incidence of curled-toe paralysis was high in all three negative control groups fed rations containing a high percentage of soybean oil meal.

The use of meat meal as the main source of protein supplement resulted in poorer growth than was obtained in any of the other experiments. The results indicate that meat meal should be used in combination with other proteins in chick starter rations if best results are to be obtained.

Summary and Conclusions

Eight experiments dealing with the replacement of buttermilk powder by substitutions involving crystalline riboflavin and protein supplements in chick starter rations are reported.

The results of these experiments are summarized in Table XVIII.

TABLE XVIII

Summary of Eight Experiments

Supplements Used	Protein Adjustment	Sex	Positive Control Av. Weight at 4 weeks in grams	Positive Control % Incidence Curled-toe Paralysis	Negative Control Av. Weight at 4 weeks in grams	Negative Control % Incidence Curled-toe Paralysis	Treatment Av. Weight at 4 weeks in grams	Treatment % Incidence Curled-toe Paralysis
(1) Meat Meal 2	Meat Meal	M	341.9		338.9		330.7	
Fish Meal 1	Fish Meal	F	305.5	0	296.3	8	298.5	0
(2) Meat Meal 1	Meat Meal							
Fish Meal 1	Fish Meal	M	336.8	0	331.1	8	329.3	0
Soybean	Soybean	F	305.1		289.3		293.0	
Oil Meal 1	Oil Meal							
(3) Meat Meal	Meat Meal	M	280.0		195.4		279.9	
		F	251.2	0	182.7	27	242.2	0
(4) Meat Meal	Fish Meal	M	278.8		253.6		285.3	
		F	242.6	0	218.6	8	258.5	0
(5) Fish Meal	Fish Meal	M	356.0		309.4		345.9	
		F	305.9	0	292.4	20	312.8	0
(6) Soybean								
Oil Meal	Meat Meal	M	318.9		223.8		291.4	
Meat Meal		F	295.5	0	213.4	38	270.6	0
(7) Soybean								
Oil Meal	Fish Meal	M	306.2		236.4		320.4	
Meat Meal		F	280.1	0	230.5	38	277.7	0
(8) Soybean								
Oil Meal	Fish Meal	M	318.7		253.3		319.5	
Fish Meal		F	295.3	0	256.4	27	300.6	0

The results of these experiments indicate that:

1. Replacement was effective where the protein combination consisted of:

(1) meat meal and fish meal in a ratio of 2:1.

(2) meat meal, fish meal and soybean oil meal in a ratio of 1:1:1.

(3) meat meal plus fish meal to replace the protein of buttermilk powder.

(4) all fish meal.

(5) a high percentage of soybean oil meal with a low percentage of meat meal plus fish meal to replace the protein of buttermilk powder.

(6) a high percentage of soybean oil meal with a low percentage of fish meal.

2. Replacement was not effective where the protein combination consisted of:

(1) all meat meal.

(2) a high percentage of soybean oil meal with a low percentage of meat meal plus meat meal to replace the protein of buttermilk powder.

III. The Stability of Riboflavin in Premixes.

Status of the Problem

The use of crystalline riboflavin in poultry rations brings up the problem of incorporation into the feed. Pure riboflavin contributes a million micrograms per gram and as a consequence it is generally necessary to add only one to two grams per ton of feed. This suggests that uniform distribution of the pure vitamin would be difficult to attain by introducing the riboflavin directly into the main feed mix.

The use of riboflavin premixes has been adopted by feed manufacturers and others to overcome this difficulty. Premixes are made by thoroughly blending the crystalline riboflavin with a small amount of finely divided feed, such as flour or wheat middlings, which are termed "carriers". The premix provides sufficient bulk to permit uniform distribution in the mixed feed. Since feed manufacturers make up large quantities of premixes which are stored over a period of time, it is important to have information regarding the stability of riboflavin in premixes.

Experimental

Riboflavin premixes were made using the following constituents as single carriers: buttermilk powder, wheat middlings, fish meal and meat meal. Sufficient crystalline riboflavin was added to increase the riboflavin potency by 350 micrograms per gram in the case of buttermilk powder, and by 250 micrograms per gram in the case of the other carriers. The riboflavin was

thoroughly mixed in a mechanical mixer for thirty minutes.

Twenty kilograms of each premix were prepared and stored in four-ply paper bags in a garage. The temperature during storage ranged from about 40°F. to 110°F. Samples of each premix were taken and assays were run in duplicate at the time the premixes were made up and at intervals for the next five months.

Results

The following table shows the results of the assays.

TABLE XIX

Assays of Riboflavin Premixes

Time	0 month	1 month	2 months	3 months	5 months
Riboflavin	γ/gm.	γ/gm.	γ/gm.	γ/gm.	γ/gm.
Fish meal	260	244	261	260	266
Meat meal	249	257	265	262	264
Wheat middlings	260	248	256	262	257
Buttermilk powder	382	379	358	371	386

Discussion

These results indicate that there was no decrease in the riboflavin content of any of the premixes over a period of five months under the conditions of storage employed.

Summary and Conclusions

Four riboflavin premixes were assayed for riboflavin after zero, one, two, three and five months of storage. No loss in riboflavin content in any of the premixes was found. Riboflavin may therefore be considered to be very stable under the conditions of storage employed in this experiment. It does not appear that loss of riboflavin in premixes during storage is likely to constitute a problem to commercial feed manufacturers.

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